

IMPROVED ORAL REHYDRATION METHODS AND COMPOSITIONS

BACKGROUND OF THE INVENTION

[001] This application claims benefit of U.S. Provisional Application serial number 5 60/416,714 filed on October 5, 2002.

Field of the Invention.

[002] In mammals, there are a variety of factors which may cause gastrointestinal distress accompanied by a loss of fluid (e.g. vomiting, diarrhea). If transient, the condition may go untreated. However, often the event can cause a fluid loss 10 accompanied by dehydration, such as in some cases of pediatric or amoebic diarrhea, necessitating intervention.

[003] Diarrhea is primarily addressed by either the administration of an agent which absorbs pathogenic bacteria, digestive enzymes, toxins, and nutrients from the gastrointestinal tract, or the administration of an oral electrolyte/sugar replacement 15 fluid, more commonly as an oral rehydration solution (ORS). In the latter approach the goal is to simply replace fluid and electrolytes that have been lost, in order to avoid dehydration and electrolyte imbalance.

[004] An electrolyte, sugar, bicarbonate solution has been the standard course of ORS therapy for the treatment of diarrhea throughout the world with the primary 20 focus being the replacement and maintenance of lost fluids. Safe and effective values of these components are well established. The gold standard as recommended by the World Health Organization (WHO) contains 20 grams glucose,

90 milliequivalents (mEq) sodium, 30 mEq bicarbonate and 20 mEq potassium per liter water.

[005] While the WHO formula is effective, it does not prevent fluid/nutrient loss but acts to replace at a rate greater than that of depletion. A means of replacing critical 5 fluids and nutrients while at the same time preventing or reducing the active rate of fluid and electrolyte loss would represent a significant improvement over current ORS therapy with widespread application in both human and animal health.

[006] Ethnomedical treatments for human disease are primarily derived from native 10 botanicals. In the case of the indigenous Amazonians, latex (sap) from the bark of *Croton* species as well as decoctions from the bark of the *Uncaria* species have both been used in the treatment of gastrointestinal distress. However, while effective, the traditional ethnomedicines have undesirable properties which limit their use.

[007] Sangre de grado, the viscous latex derived from various *Croton* species 15 plants found primarily in the Amazon River basin, is an effective agent in managing diarrhea. It is not a paralytic like loperamide but rather it works through the same mechanism as for its analgesic properties. Sensory afferent nerves drive secretory responses in the gut and preparations of sangre de grado have been shown to block epithelial secretion in response to sensory afferent nerve activation. Acute fluid shifts in response to gut injury induced by acid and undigested protein (a model of 20 bacterially driven intestinal necrosis) are blocked by preparations of sangre de grado at dilutions of 1:1000 (200 μ g/ml). In addition to preventing the secretory response, it reduces the associated damage to the intestinal mucosa. Neurally mediated epithelial secretion is characteristic of various diarrheal events, and sangre de grado

is an effective blocker of epithelial secretion of electrolytes as determined by sensory afferent nerves.

SUMMARY OF THE INVENTION

[008] Aspects of the invention are summarized below to aid in the understanding of 5 embodiment(s) of the invention and the application. Yet, the invention is fully defined by the claims of the application.

[009] An electrolyte, sugar, bicarbonate solution has been the standard course of ORS therapy for the treatment of diarrhea throughout the world with the primary focus being the replacement and maintenance of lost fluids. However, this current 10 treatment addresses the symptoms of the event but not the cause.

[010] The present invention generally comprises methods and compositions for oral rehydration in humans and animals that simultaneously reduces fluid loss. The methods and compositions disclosed herein contain botanical derivatives that retain 15 the ability to inhibit emesis and activation of sensory afferent nerves, thus reducing causative agents (i.e. vomiting, diarrhea) in combination with fluids and nutrients that address symptoms through rehydration. The methods and compositions disclosed herein incorporate botanical derivatives from the *Uncaria* and *Croton* species that retain their medicinal benefits with an electrolyte/sugar composition containing a potassium salt, a sodium salt, bicarbonate and a sugar. The invention herein relates 20 more particularly to the combination of the prior art anti-diarrheal compositions (i.e. ORS) together with the novel botanical *Uncaria* and *Croton* preparations and extracts. The purpose of these combinations with botanicals is to attenuate the

processes that promote fluid and electrolyte loss while at the same time replacing what has already been lost. It provides for a treatment that addresses both the symptoms and cause of the condition and thus offers novel benefit over current therapies by reducing the severity and duration of the conditions.

5 [011] Emesis often accompanies enteric infections, resulting in a variety of manifestations of cause for concern (i.e. dehydration, loss of medication, etc). In a well-established *in vivo* model, a novel lipid extract of the *Croton* specie *sangre de grado* (Zangrado) blocked emesis at concentrations of 3 mg/kg. Thus, extracts and preparations of *Sangre de grado* are effective anti-diarrheal agents that arrest
10 emesis and offers substantial mucosal protective properties. Cat's Claw of the *Uncaria* species, is an effective antioxidant and it has long been known that a sign of inflammation is an increased production of oxidants and free radicals. Oxidants promote diarrhea by epithelial electrolyte secretion as well as by promoting damaging gut epithelia, thereby promote a "leaky" gut barrier secondary to epithelial
15 cell death and dysfunction. A novel preparations or extracts of the *Uncaria* specie (Vincaria) prevents cell death in response to the toxic nitrogen oxide and peroxynitrite, oxidants (i.e. H₂O₂) and free radicals, implicated in gut inflammation, cell death and epithelial secretory response.

[012] According to one aspect of the invention, compositions are incorporated into
20 biologically active dosage units forming oral rehydration anti-diarrheal compositions. The final composition may be transported in a dehydrated, powdered state, in a liquid state, in a confectioned state (i.e. candy, jello, pudding, etc.) or as a food additive. If in a powdered state, some measure can be taken to prevent moisture

from causing solidification of said powder, which would interfere with fluid reconstitution (i.e. moisture-resistant packaging and/or a desiccating agent and/or crystallization). Additionally, the final compositions may also contain coloring, flavoring and sweetening agents for better palatability.

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BRIEF DESCRIPTION OF THE FIGURES

[013] FIGURE 1. The extraction process significantly ("*") reduces the proanthocyanidin content of the parent latex (SdG). When combined in a base vehicle, such as *Aloe barbadensis* shown here, the extract (CGO 110) produced a mixture absent of the intense color seen in similar preparations with the parent latex.

10 This change, which is readily quantifiable by spectrophotometer, negates the discolorizing (i.e. staining) properties commonly associated with proanthocyanidins and the parent latex and allows for practical dermatological preparations;

[014] FIGURE 2. A prototypical activator of sensory afferent nerves, capsaicin, was topically applied to the mucosal surface of the stomach in anesthetized rats and 15 mucosal blood flow measured by a Laser Doppler Flow meter. The marked increase in mucosal blood flow induced by 300 μ M capsaicin was largely prevented by either the parent material, Sangre de grado or its extract, Zangrado (CGO 110) at doses of 2 and 0.2 mg/ml, respectively, indicating that the organic extract retains the ability to effectively prevent the activation of sensory afferent nerves;

20 [015] FIGURE 3. Changes in short circuit current (measured as Isc) in guinea pig ileal mucosal preparations housed in Ussing chambers can demonstrate that sangre de grado inhibits epithelial secretion of electrolytes, as indicated by prevention of a

change in current, induced by sensory afferent nerves but not the neuropeptide Substance P. Capsaicin, the prototypical activator of these sensory afferent nerves promotes a change in short circuit current, and this is readily blocked by sangre de grado, whereas direct activation of the epithelial cells by Substance P remains 5 unaffected. This clearly establishes that sangre de grado can negate neurogenic epithelial secretion.

[016] FIGURE 4. Using a well-established ferret model of post-operative complications of nausea, emesis and itch induced by morphine, the organic extract of sangre de grado, Zangrado (CGO 110) was administered intraperitoneally 10 (3mg/kg) to ferrets 15 minutes prior to the subcutaneous injection of 0.05mg/kg of morphine-6-glucuronide (M6G). Administration of M6G caused a significant number of vomiting (2.2 ± 0.4) and retching (10 ± 1.2) incidences in the control group while in those animals pre-treated with Zangrado, the number of these episodes was virtually 15 abolished (vomiting 0.6 ± 0.3 ; retching 2.2 ± 0.7 , $P < 0.05$). It is clear that this organic extraction procedure contains active components and is effective in the treatment of emesis.

[017] FIGURE 5. A comparison of *Uncaria* parent and extract by high performance liquid chromatography (HPLC). As shown in the overlaid chromatograms, the *Uncaria* extract derived from the methods described herein (VincariaTM) is 20 substantially deplete of the immunostimulating alkaloids found in the parent (*Uncaria* spp) whilst retaining and thus enhancing the efficacy and therapeutic potential of the polar, immunosuppressive and TNF-alpha inhibiting (anti-inflammatory) components.

[018] FIGURE 6. Oxidants and free radicals can readily promote the death of epithelial cells. Here three oxidants that are structurally distinct, a free radical (DPPH), and two oxidants (peroxynitrite and hydrogen peroxide) promote death of gastric epithelial cells in culture. Inclusion of cat's claw in the form of the alkaloid-5 deplete Vincaria extract, significantly reduced cell oxidant-induced cell death, as evidence for the ability of Vincaria to maintain the integrity of the epithelial barrier.

DESCRIPTION OF EMBODIMENTS

[019] EXTRACTION PROCEDURES

10 [020] According to one aspect of this invention, an organic extraction of *Croton* species latex concentrates its lipophilic components and reduces the hydrophilic proanthocyanidin content of the plant material. The invention demonstrates that extract CGO 110 (Zangrado) reduces vomiting and diarrhea and is selectively cytotoxic to cancerous cells, unlike the parent material, representing an 15 improvement in safety and therapeutic activity.

20 [021] According to one aspect of this invention, plant material or an aqueous extract of plant materials from the *Uncaria* species are subjected to organic solvents which concentrates its polar biologically active components while reducing its lipophilic components. The invention demonstrates that the extract is effective at a dose twenty times less than extracts or decoctions produced by other methods and much less than the parent material, representing a significant improvement in therapeutic activity and potentials.

[022] According to one aspect of this invention, the extraction and removal of water through physical manipulation concentrates the biologically active components in both the *Croton* species latex and *Uncaria* species aqueous extract. The invention teaches that these extracts are more therapeutically effective at lower 5 concentrations than the parent material, representing a significant improvement in therapeutic potential and use.

[023] Preferred methods to accomplish the aforementioned species *Uncaria* and *Croton* extractions have been previously described but it is contemplated that a skilled practitioner could devise variations of the procedures given the disclosure 10 herein and the desired results.

[024] Extraction Process 1 for Croton species.

[025] Latex, or sap from *Croton* species is mixed with an organic solvent. The preferred organic solvent is ethyl acetate although other organic solvents can be used as would be obvious to the ordinarily skilled practitioner in light of the 15 disclosure herein. In other embodiments, the preferred organic solvent is isopropanol, a chloroform/Methanol mixture, or an equivalent thereof. The organic solvent is added to the latex in a 1:1 proportion. In the preferred extraction process the solvent latex combination is agitated.

[026] The preferred agitation method is stirring although other agitation methods 20 are also contemplated to be effective. Following agitation, the mixture is settled, or allowed to settle into distinct phases including at least an organic layer and an aqueous layer. The organic phase or layer is comprised largely of solute lipophilic materials, representing the active constituent, and a significantly reduced quantity of

proanthocyanidin components relative to the pre-agitation step. The organic layer is separated from the aqueous layer for further processing pursuant to the preferred extraction process.

[027] Moreover, it is common to find a gel-like substance in the organic layer at the interface of the aqueous and organic layers. This gel substance is characterized as having a dark brown and purple color and comprises hydrophilic constituents trapped with water. In the preferred process the gel substance is processed further to separate any active lipophilic constituents from the hydrophilic constituents. The preferred manner of processing the gel substance is the addition of a drying agent to the organic layer or the gel substance. The preferred drying agent is magnesium sulfate in a concentration of 0.5 – 5 g/L of contaminant gel. It is contemplated that other equivalent drying agents at relative effective concentrations would also be effective and would be obvious to the ordinarily skilled practitioner in light of the disclosure herein and with undue experimentation.

[028] The addition of the drying agent results in a precipitant, which traps water and hydrophilic constituents or water-based colored chemical contaminants. The precipitant can be readily separated from the hydrophilic constituents by filtration or other techniques known to separate precipitants. Actual laboratory procedures achieved acceptable results using a Whatman #4 filter paper or an equivalent.

[029] The steps of organic extraction, mixing with a drying agent and filtration may be repeated up to three times to accomplish a thorough extraction of the active lipophilic constituents. At this point in the process, the lipophilic materials are solutes contained within the organic solvent, which are concentrated by evaporation

of the solvent by one of several procedures, such as vacuum drying, freeze drying or heating. Actual heating up to 60 degrees Celsius produced acceptable drying results.

[030] The organic layer composition thus processed is rich in lipophilic materials but

5 largely clear of hydrophilic contaminants. Following the extraction process, the color of the organic layer can be characterized as a rose. Moreover, the reduced proanthocyanidin content is quantifiable spectrophotometrically. Relative absorbance of the extraction in the visible spectrum was compared to the absorbency peak of the parent latex (414 nm) in the visible range. At a
10 concentration of 1 mg of extracted latex to 1 mL of water the disclosed process yielding the extraction (CGO 110) results in a 4.3 fold reduction in absorbance at 414 nm, as indicated in Figure 1. This assessment was repeated 9 times with similar results achieved (significance difference $P<0.0001$, as denoted by the "*"). Similarly when sangre de grado or the extraction (CGO 110) at a concentrations of 200 μ g per
15 mL of aloe vera gel were applied to aloe vera gel to mimic their administration as topical products, there was also a significantly lower color response with the extracted sangre de grado, CGO 110 vs. the parent botanical (* $P<0.0001$). See Figure 1. Estimates from the absorbency measurements indicate that the proanthocyanidin content was reduced by at least 90% relative to the nonextracted
20 parent latex.

[031] Extraction Process 2 for the *Croton* species.

[032] The latex from the *Croton* species is dried to its residual solid matter by methods such as heating, air-drying, vacuum or freeze-drying. The dried latex is

rich in proanthocyanidin compounds and therefore characterized by a dark burgundy color. To the dried latex matter the organic solvent, ethyl acetate or an equivalent, is added. The dried latex and organic solvent mixture is agitated and the organic solvent is removed for further processing according to the procedure described in

5 Example 1. This process may be repeated up to three times to accomplish a thorough extraction all lipophilic materials in the organic layer and solvent. If any water bearing contaminants are present, the addition of drying agent followed by filtration as noted above, will remove these contaminants. Removing the ethyl acetate through various methods including heating, air-drying, vacuum or freeze-
10 drying then isolates the solutes contained within this organic extract.

[033] The extraction thus processed according to the disclosed processes is characterized by a significant reduction of proanthocyanidin compounds. The reduction of the proanthocyanidin compounds leaves the extraction significantly diminished in color producing compounds and yet amenable to health care
15 applications.

[034] Extraction Process 1 for the *Uncaria* species.

[035] An aqueous extract of the *Uncaria* species is preceded prior art decoction methods. A preferred decoction comprises a quantity of raw or dried botanical in hot water. More specifically, solid matter of the plant material such as roots, bark and or
20 powders of genus *Uncaria* are mixed in such a ratio with water that when heated for a period of time at a temperature of approximately 90-100 degrees centigrade, with or without agitation, to yield a brown aqueous extract also known as a decoction or tea. Then, according to the invention, the decoction or tea is then filtered of all solid

matter for further processing. The filtrate is subsequently dried to remove all aqueous components. Acceptable drying methods include air-drying, evaporation, or vacuum drying or an equivalent.

[036] An organic solvent is subsequently added to the dried decoction or extract. A 5 preferred organic solvent is chloroform/methanol (2:1) added in a volume-to-volume ratio of about 1:1 to 1:20. Another suitable organic solvent is ethyl acetate. Following mixing and phase settling the organic layer is removed from the aqueous layer for further processing according to one aspect of the invention. The solutes contained within the aqueous extract are then resolved by one of several drying 10 processes: heating, air drying, freeze-drying or vacuum drying. Depletion of alkaloids content in the *Uncaria* extract is confirmed by high performance liquid chromatography (HPLC) as indicated in Figure 5.

[037] Extraction Process 2 for the *Uncaria* species.

[038] An aqueous extract of the *Uncaria* species is achieved by a decoction method 15 as previously described. To this extract in its liquid phase the organic solvent ethyl acetate, is added. Following agitation and settling the organic layer is separated from the aqueous and the organic layer discarded. The solutes remaining in the aqueous layer are the resolved by drying. Several drying processes can be used: heating, air-drying, freeze-drying, or vacuum drying. Depletion of alkaloids content 20 in the *Uncaria* extract is confirmed by high performance liquid chromatography (HPLC) as indicated in Figure 5.

[039] Extraction Process 3 for the *Uncaria* species.

[040] This process employs the same decoction extraction method as described for Examples 1 and 2, but before the extraction of organic material is employed; the solute in the decoction is isolated by drying. To this dried powder the organic solvents are added (chloroform/methanol, 2:1: or ethyl acetate). This mixture is 5 agitated for adequate mixing, followed by settling. The liquid organic solvent is removed and discarded, and the solutes are dried again as outlined by similar methods employed for drying in Example 1 or 2. Depletion of alkaloids content in the *Uncaria* extract is confirmed by high performance liquid chromatography (HPLC) as indicated in Figure 5.

10 [041] COMPOSITIONS

[042] According to one aspect of this invention, the mixture of sugars, minerals and fluids in a fixed ratio and commonly known as an oral rehydration solution (ORS) can be combined with *Croton* species extracts (Zangrado) herein described. The invention demonstrates that the ORS-Zangrado combination (ZORS) is much more 15 effective at treating gastrointestinal events characterized by a loss of fluid than current ORS therapy, Zangrado, other *Croton* extracts or the parent material alone. According to another aspect of the invention, the mixture of sugars, minerals and fluids in a fixed ratio (ORS) can be combined with *Croton* species extracts (Zangrado) and *Uncaria* species extracts (Vincaria) herein described. The invention 20 demonstrates that the ORS-Zangrado-Vincaria combination (V-ZORS) is more effective at treating gastrointestinal events characterized by a loss of fluid than either current ORS therapies, Zangrado or other *Croton* extracts, Vincaria or other *Uncaria* extracts or the parent materials alone.

[043] EFFECTS OF ZANGRADO ON SENSORY AFFERENTS

[044] Sensory afferent nerves mediate the sensations of pain, itch, cough and nausea and when activated by capsaicin, lead to a multitude of responses including vasodilation, inflammation, edema, and pain and itching. Using a well-established rat 5 model, the mucosal surface of the stomach in anesthetized animals was inoculated with 300 μ M capsaicin and mucosal blood flow measured by a laser Doppler flow meter.

[045] As indicated in Figure 2, the marked increase in capsaicin-induced mucosal blood flow was prevented by either the sangre de grado parent material (SdG) or its 10 organic extract Zangrado (CGO 110) at doses of 2 and 0.2 mg/ml, respectively. Thus, the sangre de grado organic extract described in this application retains the ability to effectively prevent the activation of sensory afferent nerves and offers significant benefit over the parent material. Evidence that Sangre de grado affects 15 neurally-mediated electrolyte secretion is depicted in Figure 3. Changes in capsaicin-induced short circuit current are attenuated by Sangre de grado, demonstrating that the locus of action is neurally mediated secretory events.

[046] EFFECTS OF ZANGRADO ON MORPHINE-INDUCED EMESIS AND ITCH

[047] Using a well-established ferret model of post-operative complications of nausea, emesis and itch induced by morphine, the extraction Zangrado (CGO 110) 20 was administered intraperitoneally (3mg/kg) to ferrets 15 minutes prior to the administration of morphine-6-glucuronide (M6G), known to promote itching, retching and vomiting. The animals were monitored for sixty minutes.

[048] As shown in Figure 4, the M6G caused a significant number of vomiting (2.2 ± 0.4) and retching (10 ± 1.2) incidences in the control group while in those animals treated with extract Zangrado, the number of episodes was virtually abolished (vomiting 0.6 ± 0.3 ; retching 2.2 ± 0.7 , $P < 0.05$). Itch as indicated by licking 5 responses was reduced from a control value of 16.9 ± 2.3 episodes to 2.2 ± 0.7 in Zangrado treated animals ($P < 0.05$). Thus, the extract offers significant improvement over the parent material or other preparations in the treatment of nausea and emesis.

[049] INHIBITION OF TNF α FORMATION

10 [050] The extract of *Uncaria* species processed according to the invention produced a composition with an enhanced ability to inhibit the formation of tumor necrosis factor alpha (TNF α) by macrophages stimulated by bacterial endotoxin [lipopolysaccharide (LPS)]. This activity is superior to that of other preparations or the parent material and therefore offers significant benefit in the treatment of 15 gastrointestinal distress.

[051] **Table 1: Antioxidant and anti-TNF α actions of cat's claw formulations.**

Note that in order to assess activity in micropulverized *Uncaria*, a hot water extraction was performed and this decoction used for evaluation. TNF α production was assessed in cultured macrophages (RAW 264.7 cells) stimulated with bacterial 20 endotoxin (LPS: $0.5 \mu\text{g/mL}$). After one hour exposure to LPS, media was collected and TNF α levels measured by ELISA. DPPH scavenging was spectrophotometrically measured by a reduction in absorbance at 515 nm. Results are depicted as IC₅₀, which is the concentration that produces a 50% inhibition. A lower IC₅₀ value is

indicative of greater potency. Note: For each assay, potency was significantly greater in alkaloid depleted freeze-dried formulation when compared to the other formations (P<0.01)

[052] Table 1

Assay IC ₅₀	Freeze-dried Alkaloid Deplete Uncaria (Vincaria™)	Freeze-dried Uncaria Alkaloid Intact	Micropulverized Uncaria Alkaloid Intact
DPPH	12.6 µg/mL	20.8 µg/mL	150 µg/mL
Anti-TNF α	9.5 ng/mL	14.1 ng/mL	28 ng/mL

5 [053]

[054] PREVENTION OF OXIDANT-MEDIATED EPITHELIAL CELL DEATH

[055] The extract of *Uncaria* species was found to be a very effective inhibitor of epithelial cell death induced by structurally divergent oxidants and free radicals (Figure 6). Epithelial cell death leads to fluid and electrolyte loss as a consequence 10 of a loss of the integrity of the barrier function. Inclusion of cytoprotective components in a standard ORS solution represents an improvement in the approach to reduce the duration and severity of the diarrheal symptoms.

[056] Although the invention has been described in detail with reference to one or more particular preferred embodiments, persons possessing ordinary skill in the art 15 to which this invention pertains will appreciate that various modifications and enhancements may be made without departing from the spirit and scope of the claims that follow.